

Docket No.: 20269/1201776-US2
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Jay D. KRANZLER et al.

Application No.: 10/623,431

Art Unit: 1614

Filed: July 18, 2003

Examiner: Rebecca COOK

For: METHODS OF TREATING FIBROMYALGIA
SYNDROME, CHRONIC FATIGUE
SYNDROME AND PAIN

DECLARATION OF DR. K. RANGA RAMA KRISHNAN UNDER 37 C.F.R. § 1.132

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, K. RANGA RAMA KRISHNAN, do hereby declare and state the following:

1. I, K. Ranga Rama Krishnan, am a citizen of the United States, and I am more than twenty-one years of age.
2. I make this declaration in support of this application ("the '431 application").
3. I received a Bachelor's degree in Medicine and Surgery from Madras Medical College at the University of Madras in 1978. I was a Psychiatry Resident at Duke University Medical Center from 1981-83, and a Fellow in Neurobiology at Duke University Medical Center from 1982-84.

Application Serial No. 10/623,431

Attorney Docket No. 20269/1201776-US2

4. I presently hold the position of Chairman of Psychiatry at Duke University Medical Center, Durham, North Carolina and have held this position for seven years. My present positions at Duke also include Professor of Psychiatry and Director, Affective Disorders Unit. My prior positions at Duke include Head, Division of Biological Psychiatry; Associate Professor of Psychiatry; and Clinical Associate Physician.

5. In addition to treating psychiatric patients, I have been involved in research in the field of psychopharmacology for twenty years. I have published more than 275 articles relating to psychopharmacology in peer reviewed journals.

4. My qualifications as a scientist, and in particular in the field of psychopharmacology, are set forth in more detail in my curriculum vitae, which is attached as Exhibit A.

5. I have read and am familiar with the specification of the '431 application, the Office Action mailed March 18, 2005, the Response to Office Action filed June 10, 2005, the Office Action mailed August 19, 2005, and the published international patent application WO 00/32178 ("the '178 application").

6. It is my understanding that the Examiner believes that a dual serotonin norepinephrine reuptake inhibitor compound (SNRI) must necessarily inhibit the reuptake of dopamine because sibutramine can be used to treat the same conditions (i.e., the "pain of CFS and FMS") as a SNRI (Office Action mailed August 19, 2005, page 2).

7. It is my understanding that the Examiner based her finding that sibutramine can be used to treat pain of CFS and FMS on the disclosure of the '178 application (see Office Action mailed August 19, 2005, page 2).

8. Sibutramine is a triple reuptake inhibitor, which inhibits the reuptake of dopamine, serotonin and norepinephrine. See, for example, the '178 application at page 9, lines 29-31.

9. The '431 application defines SNRIs as a "well-recognized class of anti-depressant compounds that selectively inhibit reuptake of both serotonin and norepinephrine" ('431 application, page 4, lines 25-27).

10. The '431 application provides examples of common SNRI compounds including venlafaxine, duloxetine and milnacipran ('431 application, page 4, lines 27-28).

11. The term SNRI is well-known and understood to medical personnel including, for example, medical doctors and researchers in the pharmaceutical arts as referring to a compound that selectively inhibits the reuptake of serotonin and norepinephrine.

12. It is well known in the field of psychiatric medicine that a SNRI compound is distinct from a triple reuptake inhibitor compound such as sibutramine because a SNRI compound does not inhibit the reuptake of dopamine. This has been well known to me and to the psychiatrists in my department at Duke for more than 20 years.

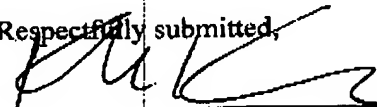
13. There are also primarily norepinephrine reuptake inhibitors that do not inhibit dopamine reuptake. The transporters for dopamine and norepinephrine are distinct. This has been well known to me and to the psychiatrists in my department at Duke for more than 20 years.

14. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true. I further declare that these statements are made with the knowledge that the willful false statements and the like

so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States code, and that such willful false statements may jeopardize the validity of the instant application or of any patent issued thereupon.

11/15/05
DATE

Respectfully submitted,


K. Ranga Rama Krishnan, MBBS

Curriculum Vitae

Personal Information

Name: K. Ranga Rama Krishnan Social security number: 240-35-0391

Home Address: 104 Huntington Drive, Chapel Hill NC 27514

Work Address: Box 3950, Duke Medical Center, Durham, NC 27710

Work Phone: (919) 684-5616 Fax: (919) 681-5489

Date & Place of Birth: April 22, 1956 Madras, India

Marital Status: Married

Education

High School: Madras Christian College School, 1972

College: Loyola College, University of Madras, 1973

Graduate: Madras Medical College,
University of Madras, 1978, Bachelor of Medicine and Surgery
MBBS, 1978

Employment History

Chairman of Psychiatry, July 1998 to present

Professor of Psychiatry, June 1995 to present.

Head, Division of Biological Psychiatry, 1992 to 2000.

Director, Affective Disorders Program, 1992 to present.

Associate Professor of Psychiatry, 1990 to 1995

Medical Director, Affective Disorders Unit, 1989-1992

Assistant Professor of Psychiatry, 1984-1989.

Clinical Associate Physician, Clinical Research training on Clinical Research Unit, 1985-1987.

Fellow in Neurobiology, Duke University Medical Center, 1982-1984.

PG4Y Resident, Department of Psychiatry, Duke University Medical Center. 1983-1984

Chief Resident in Psychiatry, Duke-Umstead Program, 1982-1983.

Residency: Duke University - John Umstead Program, 1981-1983.

Senior House Officer, Acting Registrar: Department of Emergency Medicine, Institution: Queen Elizabeth Hospital, University of West Indies, Barbados, West Indies, 1980-1981.

Rotating Internship: Institute of Obstetrics and Gynecology; Government General Hospital, Department of Internal Medicine, Department of Surgery; Institute of Child Health; Department of Community and Preventive Medicine. Madras Medical College, 1979-80.

Certification/Licensure

Tamilnadu Medical Council, India	1979
Medical License, Barbados, West Indies	1980
ECFMG Certification	1980
Texas Board of Medical Examiners (FLEX)	1981
North Carolina Board of Medical Examiners	1983 (26679)
Georgia Board of Medical Examiners	1987
National Board of Medical Examiners, Diplomate	1996

Specialty certification and dates:

Psychiatry, 1986

Certified in Geriatric Psychiatry, American Board of Psychiatry and Neurology, 1991.

Controlled Substances Registration Certification- Practitioner 1996

Awards

Laughlin Award, American College of Psychiatry, 1984

Collegium Internationale Neuro-Psychopharmacologicum Rafaelsen Fellowship Award, 1988.

Walker P. Inman Memorial Fund Grant for a study of extrapituitary regulation of cortisol, 1984.

Clinical Associate Physician Award, General Clinical Research Center Program, 1985-1987.

Dorfman Award, Best Paper in Psychosomatics, 1990.

Listed in Marquis' "Who's Who in Science and Engineering, 1996.

NARSAD Distinguished Investigator Award for Genetic Imaging Study in Bipolar Disorder granted by National Alliance for Research on Schizophrenia and Depression (NARSAD) 1996-98.

Center for the Study of Aging & Human Development, Senior Fellow, 1999-

Honored Teacher Award, Department of Psychiatry & Behavioral Sciences, Duke University Medical Center, 2001.

Present Appointments:

1998 - date Chairman, Department of Psychiatry and Behavioral Sciences, Duke University Medical Center, Durham, North Carolina.

1995 - date Professor, Department of Psychiatry and Behavioral Sciences, Duke University Medical Center, Durham, North Carolina.

1992 - date Head, Division of Biological Psychiatry, Duke University Medical Center, Durham, North Carolina

1992 - date Director, Affective Disorders Program, Duke University Medical Center, Durham, North Carolina

Previous Appointments:

Associate Professor of Psychiatry, 1990 to 1995

Medical Director, Affective Disorders Unit, 1989-1992

Assistant Professor of Psychiatry, 1984-1989.

Clinical Associate Physician, Clinical Research training on Clinical Research Unit, 1985-1987.

Fellow in Neurobiology, Duke University Medical Center, 1982-1984.

PG4Y Resident, Department of Psychiatry, Duke University Medical Center. 1983-1984

Chief Resident in Psychiatry, Duke-Umstead Program, 1982-1983.

Residency: Duke University - John Umstead Program, 1981-1983.

Senior House Officer, Acting Registrar: Department of Emergency Medicine, Institution: Queen Elizabeth Hospital, University of West Indies, Barbados, West Indies, 1980-1981.

Rotating Internship: Institute of Obstetrics and Gynecology; Government General Hospital, Department of Internal Medicine, Department of Surgery; Institute of Child Health; Department of Community and Preventive Medicine. Madras Medical College, 1979-80.

Professional Societies:

American Psychiatric Association

International Association for the Study of Pain

Society of Biological Psychiatry

American Association for the Advancement of Science

The New York Academy of Sciences

International Society of Psychoneuroendocrinology

The American Geriatric Society

Collegium Internationale Neuropsychopharmacologicum (CINP)

American College of Neuropsychopharmacology (ACNP)

The American College of Forensic Examiners, Psychiatry Division, 1996.

Professional Service:

Editorial Board Member, Behavioral Medicine
Editorial Board Member, Psychiatry Research: Neuroimaging
Editorial Board Member, Depression
Editorial Board Member, Biological Psychiatry
Editorial Board Member, Psychopharmacology Bulletin
Editorial Board Member, Neuropsychopharmacology

Reviewer for the following journals:

Psychosomatic Medicine
Archives of General Psychiatry
Pain
Psychoneuroendocrinology
Psychiatry Research
International Journal of Geriatric Psychiatry
American Heart Journal
American Journal of Psychiatry

Consultant/Review Panels:

U.S. Veterans Administration, Review Board in Endocrinology (Ad Hoc), 1988.

Member, Subcommittee DSM IV Panel for Impulse Control Disorders, 1988 to 1993.

Program Committee, Society of Biological Psychiatry Meeting, Montreal, 1988.

U.S. Veterans Administration, Review Board in Psychiatry (Ad Hoc), 1988-89.

N.I.A.A.A. (Ad Hoc) Grant Review and Site Visitor, Special Review Committee, 1991, 1993.

U.S. Veterans Administration, Review Board in Career Development Program, 1992.

N.I.M.H. (Ad Hoc) Grant Review and Site Visitor, Special Review Committee, 1986, 1990, 1994; 1995, 1996.

Consultant, Longitudinal Bipolarity in 6-17 year olds: Genetics, MRI, ERP (Dr. Barbara Geller and Dr. John Rohrbaugh, Co-PI) Washington University School of Medicine, St. Louis, 1992 to 1996.

Consultant, Brain imaging in schizophrenia (MIND) (Dr. Keshevan PI) University of Pittsburgh Medical Center, 1996-1998.

Consultant and member Steering Committee, Enhancing Recovery in Coronary Heart Disease (ENRICH). National Heart, Lung, and Blood Institute National Institutes of Health, Department of Health & Human Services, 1995 to present.

Consultant and member Advisory Panel, Mental Health Clinical Research Center for the Study of Schizophrenia, (Dr. John Kane, PI) Albert Einstein College of Medicine, New York, 1995 to present.

Consultant/Review Panels: cont'd:

Consultant, Clinical Mental Health Academic Award in Geriatric Psychiatry: Brain Morphometry in Geriatric Depression, Department of Psychiatry, Long Island Jewish Medical Center (Dr. Blaine Greenwald, PI), New York, 1996-1997.

Consultant, NIH Late Life Depression (Dr. Harold Sackheim, PI) New York State Psychiatry Institute, New York, 1995-1996.

Member, TDA-II Psychopharmacology Review Committee National Institute of Mental Health, 1990 to 1992.

Member, Biological Psychopathology Review Committee National Institute of Mental Health, 1992 to 1993.

Member, Psychobiological, Biological, and Neurosciences Subcommittee, Mental Health Acquired Immunodeficiency Syndrome Research Review Committee, National Institute of Mental Health, 1993 through June 1995.

Member, National Board of Medical Examiners USMLE Step 1 Test Material Development Committee for Behavioral Sciences and the National Board's Behavioral Sciences Test Committee, 1994-1997.

Member, National Institute of Mental Health, Clinical Neuroscience & Biological Psychopathology Review Committee, 1995-1998

Member, Special Emphasis Panel, Clinical Neuroscience Review Committee, National Institutes of Health, 1996.

Duke University Medical Center:

Chairman, Private Diagnostic Clinic Advisory Board Committee, Dept. of Psychiatry & Behavioral Sciences, 1996 to present.

Member, Search Committee for Director of the Brain Imaging and Analysis Center, 1996.

Member, Provost Planning Group on Cognitive Neuroscience, 1996.

Member, Psychiatry Steering Research Committee, 1992 to present.

Member, Psychiatry Research Space Committee, 1992 to 1994.

Consultant to Pain Program, 1984 to present.

Grants Received:**Federal Grants:**

Co-Investigator, NIMH Grant #5R01 MA 39593-04, "Neuroendocrine Regulation in Depression", Bernard J. Carroll, M.D., Ph.D., 1987-1991.

Co-Investigator, NIMH Grant #P50 MH40159-05, "CRC/PE For the Study of Depression", Dan G. Blazer, M.D., Ph.D., (1984-1993); Bernard J. Carroll, M.D., Ph.D., July 1994 through August 1999.

Principal Investigator, NIMH Grant, "Stress, Cortisol, Cognition, Brain Atrophy and Depression", MH447166-01, 1989-1994. 1994 - 1998.

Grants Received:**Federal Grants: cont'd.**

Co-Principal Investigator, NIMH Grant #R01 MH045975-03, "TRH in thermoregulation and depression: basic and clinical studies." 1989 to 1991.

Co-Principal Investigator, NIMH Grant, "Victims of violence: family risk for PTSD following rape." 1991 to 1994.

Co-Principal Investigator, NIMH Grant #5R01 MH049679, "Exercise training and depression in older adults." P.I. Jim Blumenthal, Ph.D. 1994 to 1998.

Principal Investigator, NIMH Grant #R01-MH051191-08, "Depression in elderly cardiac patients." 1994 to 1998 and 1998 to 2003

Principal Investigator, NIH National Institute on Aging Grant #R01-AG011913-03, "Alzheimer's disease: Antemortem markers." 1994 to 1998.

Co-Principal Investigator, NIMH Grant #SP30 MH40159, "Clinical Research Center for the study of depression in the elderly." 1994 to 1999.

Principal Investigator, NIMH Grant #5P30 MH40159, "Clinical Research Center for the study of depression in the elderly." 1997 to 1999.

Principal Investigator, NIMH Grant #R01-MH050570-05, "Depression in late life." 1996 to 2002.

Principal Investigator, NIMH Grant #R01-MH057027-05, "Bipolar Disorder in late life." 1998 to 2003.

Co-Investigator, NIMH Grant UNC#5-32857, "Neuroimaging in HIV infection" 1997 to 2002.

Co-Principal Investigator, National Heart, Lung, and Blood Institute National Institutes of Health, Department of Health & Human Services Grant, Enhancing Recovery in Coronary Heart Disease (ENRICHED), 1995 to present.

Principal Investigator, NIMH Grant #P50-MH060451-03, "Conte Centers for the Neuroscience of Depression." 2001 to 2006.

Principal Investigator, NIMH Grant #R01-MH063211-02, "Safety and Efficacy of Sertraline for Depression CHF. 2003 to 2008.

Co-Principal Investigator, NIH Grant #M01-RR003-0 "General Clinical Research Center." 2001 to 2003.

Principal Investigator, NIMH Grant #R01-MH63211-01, "Inflammatory Biomarkers in Depressed CHF Patients. 2003-2008.

Principal Investigator, NIMH Grant #P30-MH040159-14, Study of Depression.

Co-Investigator, NIMH Grant #R01-MH054846-03 Geriatric Depression--Risk Factors for Adverse Outcome.

Principal Investigator, NIMH Grant #R01-MH044716-06. Cortisol Cognition Brain Atrophy and Depression.

Industry Grants:

Co-Investigator, Safety Study Maprotiline in Depression, Ciba-Geigy, 1984.

Co-Investigator, Open Label Trial of Gepirone in Depression. Bristol-Myers, 1986.

Principal Investigator, "A 52-week, randomized, double-blind, placebo-controlled, parallel-group, multicenter study of Milameline (CI-979/RU 35926) in patients with probable Alzheimer's disease with long-term open-label extension." Parke-Davis Pharmaceutical Research July 1996 - July 1997.

Principal Investigator, "Sertraline in management of patients with chest pain despite normal coronary angiograms." Pfizer U.S. Pharmaceuticals July 1996 - July 1997.

Principal Investigator, "A multicenter, parallel-group, double-blind, placebo-controlled study of the efficacy, tolerability and safety of Sertraline in the treatment of the behavioral manifestations of Alzheimer's disease in outpatients. Pfizer U.S. Pharmaceuticals August 1996 - August 1997.

Investigator-Initiated IND:

1. Ovine CRF Corticotrophin-Releasing Factor
2. RU-486 in Depression

1. Selvaraj AM, Mallika K, Krishnan KRR: Spontaneous Aerogram of the Biliary Tree. Antiseptic (India), 1980.
2. Krishnan KRR, Maltbie AA, Davidson JRT: Abnormal Cortisol Suppression in Bipolar Patients with Simultaneous Manic and Depressive Symptoms. Am J Psychiatry 140:203-205, 1983.
3. Varia IM, Krishnan KRR, Davidson JRT: Deep Vein Thrombosis as a Complication of Antipsychotic Therapy. Psychosomatics 24:1097-1098, 1983.
4. Krishnan KRR, Volow MR, Cavenar JO, Jr., Miller PP: Dreams of Flying in Narcoleptic Patients. Psychosomatics 25:423-425, 1984.
5. Krishnan KRR, Volow MR, Miller PP: Narcolepsy: Preliminary Retrospective Study of Psychiatric and Psychosocial Aspects. Am J Psychiatry 141:428-431, 1984.
6. Krishnan KRR, France RD, Ellinwood EH, Jr: Tricyclic Induced Akathisia in Patients Taking Conjugated Estrogens. Am J Psychiatry 141:696-697, 1984.
7. Krishnan KRR, Davidson JRT, Miller RD: MAO Inhibitor Therapy in Trichotillomania Associated with Depression: A Case Report. J Clin Psychiatry 45:310-311, 1984.
8. Krishnan KRR, Swartz M, Larson MR, Santoliquido G: Funeral Mania in Recurrent Bipolar Affective Disorders: Reports of Three Cases. J Clin Psychiatry 45:310-311, 1984.
9. Carrington CD, Ellinwood EH, Jr., Krishnan KRR: Effects of Single and Repeated Alcohol Withdrawal on Kindling. Biol Psychiatry 19:525-537, 1984.
10. Krishnan KRR, Davidson JRT, Rayasam K: Dexamethasone Suppression Test in Borderline Personality Disorder. Biol Psychiatry 19:1149-1153, 1984.
11. Davidson JRT, Lipper S, Zung WWK, Strickland R, Krishnan KRR, Mahorney S: DST as an External Validation of Different Diagnostic Criteria. Am J Psychiatry 141:1221-1223, 1984.
12. France RD, Krishnan KRR, Houpt JL, Maltbie AA: Differentiation of Depression from Chronic Pain Using the DST and DSMIII. Am J Psychiatry 141:1577-1579, 1984.
13. Krishnan KRR, Davidson JRT, Guajardo C: Trichotillomania: A Review. Comprehensive Psychiatry 26:123-128, 1985.
14. Krishnan KRR, France RD and Houpt JL: Chronic low back pain and depression. Psychosomatics 26:299-304, 1985.
15. Krishnan KRR, France RD, Pelton S, McCann UD, Manepalli AN and Davidson JRT: What Does the DST Identify? Biol Psychiatry 20:957-964, 1985.
16. Krishnan KRR, France RD, Pelton S, McCann UD, Davidson JRT, and Urban BJ: Chronic Pain and Depression. Part I - Classification of depression in chronic low back pain patients. Pain 22:279-287, 1985.
17. Krishnan KRR, France RD, Pelton S, McCann UD, Davidson J and Urban BJ: Chronic Pain and Depression. Part II - Symptoms of anxiety in chronic low back pain patients and their relationship to subtypes of depression. Pain 22:289-294, 1985.

35. Goldstein D, Gockerman J, Krishnan R, Ritchie J, Tsy CY, Hood LE, Ellinwood EHJr, Laszlo J: Effects of gamma interferon on the endocrine system: results from a phase I study. *Cancer Res* 47:6397-6401, 1987.
36. Krishnan KRR, Manepalli AN, Ritchie JC, Rayasam K, Melville ML, Rivier J, Vale W, Thorner MO, Nemeroff CB: Growth Hormone Response to GHRF in depression. *Peptides* 9:113-116, Supplement 1, 1987.
37. France RD, Urban B, Krishnan KRR, Bissette G, Banki CM, Nemeroff CB: CSF corticotropin-releasing factor-like immunoactivity in chronic pain patients with and without major depression. *Biol Psychiatry* 23:86-88, 1988.
38. Krishnan KRR, Manepalli AN, Ritchie JC, Rayasam K, Melville ML, Rivier J, Vale W, Thorner MO, Carroll BJ, Nemeroff CB: GHRF stimulation test in depression. *Am J Psychiatry* 145:90-92, 1988.
39. Krishnan KRR, Goli V, Ellinwood EH, France RD, Blazer DG, Nemeroff CB: Leukoencephalopathy in patients diagnosed as major depression. *Biol Psychiatry* 23:519-522, 1988.
40. France RD, Skott A, Krishnan KRR, Urban B, Houpt, J: Subtypes of depression in patients with chronic pain. *Southern Medical Journal* 81:485-488, 1988.
41. Krishnan KRR, Ritchie JC, Manepalli AN, Nemeroff CB, Carroll BJ: Adrenocortical sensitivity to ACTH in man. *Biol Psychiatry* 23:86-88, 1988.
42. Naylor MR, Krishnan KRR, Manepalli AN, Ritchie JC, Wilson WH, Carroll BJ: Circadian rhythm of adrenergic regulation of ACTH and cortisol secretion in man. *J Clin Endocrin & Metab* 67:404-406, 1988.
43. Heyman A, Schmechel D, Wilkinson W, Rogers H, Krishnan R, Holloway D, Schultz K, Gwyther L, Peoples R, Utley C and Haynes C: Failure of long term high dose lecithin to retard progression of early onset Alzheimer's disease. *J Neural Transmission* 24:279-286, 1988.
44. Davidson JRT, Woodbury M, Pelton S, Krishnan R: A study of depressive typologies using grade of membership analysis. *Psychological Medicine* 18:179-189, 1988.
45. Nemeroff CB, Knight DL, Krishnan KRR, Slotkin TA, Bissette G, Melville ML, Blazer D: Marked reduction in the number of platelet 3H-imipramine binding sites in geriatric depression. *Arch Gen Psychiatry* 45:919-923, 1988.
46. Krishnan KRR, Heyman A, Ritchie JC, Utley CM, Dawson DB, Rogers H: Depression in Early-Onset Alzheimer's Disease: Clinical and Neuroendocrine Correlates. *Biol Psychiatry* 24:937-940.
47. Heinz R, Rayasam K, Krishnan KRR, Wingfield M, Irigaray P, Ellinwood EHJr: MRI scans in patients with tardive dyskinesia. *Biol Psychiatry* 24:852-857, 1988.
48. Slotkin TA, Whitmore WL, Barnes GA, Krishnan KRR, Blazer D, Knight DL, Nemeroff CB: Reduced inhibitory effect of imipramine on radio-labeled serotonin uptake into platelets in geriatric depression. *Biol Psychiatry* 25:687-691, 1989.
49. Krishnan KRR, France RD: Antidepressants in the Management of Chronic Pain Syndromes. *Am Family Physician* 30:4:233-237, 1989.
50. Figiel GS, Krishnan KRR, Nemeroff CB, Breitner JC: Radiologic correlates of antidepressant induced delirium. *J Neuropsychiatry Clin Neurosci* 1:2:188-190, 1989.

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67. Breitner JCS, Husain MM, Figiel GS, Krishnan KRR, Boyko OB. Cerebral white matter disease in late-onset paranoid psychosis. *Biol Psychiatry* 28(3): 266-274, 1990 .
68. Nemeroff CB, Bissette G, Owens MJ, Vargas MA, Pihoker C, Krishnan KRR, Cain ST and Banki CM. Corticotropin-releasing factor: preclinical and clinical studies. *Clin Neuropharmacol Suppl* 2(13): 595-596, 1990.
69. McDonald WM, Husain M, Doraiswamy PM, Figiel GS, Boyko O, Krishnan KRR. A magnetic resonance image study of age-related changes in human putamen nuclei. *NeuroReport* 2(1): 57-60, 1991.
70. Na CA, Doraiswamy PM, HK. Lee, Krishnan KRR. MRI in biological psychiatry *Prog in Neuro-Psychopharm & Biol Psychiat* 15:581, 1991.
71. Krishnan KRR, Ritchie JC, Manepalli AN, Saunders WB, Li SW, Venkataraman S, Nemeroff CB, Carroll BJ. Fast feedback regulation of ACTH by cortisol. *Prog Neuro-Psychopharm & Biol Psychiat* 15(4): 523-529, 1991.
72. Figiel GS, Botteron K, Zorumski CF, Jarvis MR, Doraiswamy M, Krishnan KRR. the treatment of late age onset psychoses with electroconvulsive therapy. *International J Geriatric Psychiatry* 4: 44-57, 1991.
73. McDonald WM, Krishnan KRR, Doraiswamy PM, Figiel GS, Husain MM, Boyko OB, Heyman A. Magnetic resonance findings in patients with early-onset Alzheimer's disease. *Biol Psychiatry* 29(8): 799-810, 1991.
74. Krishnan KRR, Doraiswamy PM, Lurie SN, Figiel GS, Husain MM, Boyko OB, Ellinwood EH Jr, Nemeroff CB. Pituitary size in depression. *J Clin Endocrin & Metab* 72: 256-259, 1991.
75. Husain MM, Doraiswamy PM, Knight DL, Boyko OB, Krishnan KRR, Nemeroff CB. Platelet [3-H]-imipramine binding and leukoencephalopathy in geriatric depression. *Biol Psychiatry* 29(7): 655-670, 1991.
76. Figiel GS, Krishnan KRR, Roa VP, Doraiswamy PM, Boyko OB, Nemeroff CB. Subcortical hyperintensities on brain MRI: A comparison of normal and bipolar subjects. *J Neuropsych Clin Neurosci* 3:18-22, 1991.
77. Krishnan, KRR. Organic bases of depression in the elderly. *Annual Review Medicine* 42: 261-66, 1991.
78. Figiel GS, Krishnan KRR, Doraiswamy PM, Rao VP, Nemeroff CB, Boyko OB. Subcortical hyperintensities on brain magnetic resonance imaging: A comparison between late age onset and early onset elderly depressed subjects. *Neurobiol of Aging* 12(3): 245-247, 1991.
79. Churchill CM, Priolo CV, Nemeroff CB, Krishnan KRR and Breitner JCS. Occult subcortical magnetic resonance findings in elderly depressives. *International J Geriatric Psychiatry* 6: 213-216, 1991.
80. Gupta SK, Krishnan KRR, Ellinwood EH, Ritchie JC and Nemeroff CB. Pharmacokinetics of human growth hormone secretion in humans induced by growth hormone releasing hormone. *Life Sciences* 47: 1887-1893, 1991.
81. Escalona PR, McDonald WM, Doraiswamy PM, Boyko OB, Husain MM, Figiel GS, Laskowitz D, Ellinwood EH Jr, Krishnan KRR. *in vivo* stereological assessment of human cerebellar volume: Effects of gender and age. *Amer J Neuroradiol* 12(5): 927-929, 1991.

82. Goli V, Krishnan KRR and Ellinwood EH Jr. Obsessive compulsive disorder. *Canadian Family Physician*. 37:1449-1455, 1991.
83. Doraiswamy PM, Krishnan KRR, Husain MM, Boyko, OB, Figiel GS, Palesi VJ, Shah SA, McDonald WM, Rockwell WJK, Ellinwood EH Jr. Pituitary abnormalities in eating disorders: Further evidence from MRI studies. *Prog Neuro-Psychopharmacol & Biol Psychiatr* 15(3): 351-356, 1991.
84. Krishnan KRR, Manepalli AN, Ritchie JC, Venkataraman S, Wilson WH, Carroll BJ. Role of serotonin in HPA axis escape from dexamethasone suppression. *Prog Neuro-Psychopharmacol & Biol Psychiatr* 15: 637-642, 1991.
85. Doraiswamy PM, Figiel GS, Husain MM, McDonald WM, Shah SA, Boyko OB, Ellinwood EH Jr, Krishnan KRR. Aging of the human corpus callosum: Magnetic resonance imaging in normal volunteers. *J Neuropsychiatry & Clin Neurosci* 3(4): 392-397, 1991.
86. Potts NS, Davidson JRT, Krishnan KRR, Doraiswamy MP, Ritchie J. Urinary free cortisol levels in social phobia. *J Clin Psychiatry* 52: 41-42, 1991.
87. Na C, Doraiswamy PM, Lee KH, Krishnan KRR. Magnetic resonance imaging in biological psychiatry. *Prog Neuro-Psychopharmacol & Biol Psychiatr* 15(5): 581-593, 1991.
88. Davidson JRT, Potts NS, Richichi EA, Ford SM, Krishnan KRR, Smith RD, Wilson WIF. The brief social phobia scale. *J Clin Psychiatry*, 11: 48-51, 1991.
89. Figiel GS, Hassen MA, Zorumski CF, Krishnan KRR, Doraiswamy PM, Jarvis MR, Smith, DS. ECT-induced delirium in depressed patients with Parkinson's disease. *J Neuropsychiatry & Clin Neurosci* 3(4): 405-411, 1991.
90. Husain MM, McDonald WM, Doraiswamy PM, Na C, Figiel GS, Escalona PR, Boyko OB, Nemeroff CB, Krishnan KRR. A magnetic resonance imaging study of putamen nuclei in major depression. *Psychiatry Research: Neuroimaging* 40(2): 95-100, 1991.
91. Shah SA, Doraiswamy PM, McDonald WM, Husain MM, Escalona PR, Figiel GS, Boyko OB, Nemeroff CB, Ellinwood Jr., EH, Krishnan KRR. Assessment of posterior fossa structures with midsagittal MRI: The effects of age. *Neurobiology Aging* 12: 371-374, 1991.
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Monoamine Reuptake and NMDA Antagonist Profile of Milnacipran: A Comparison to Duloxetine

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Introduction

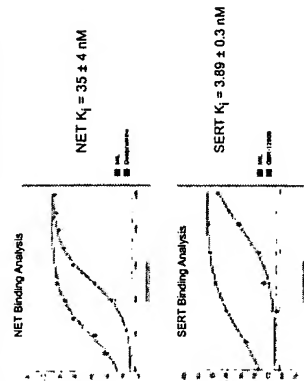
Milnacipran (MIL) is a norepinephrine (NE)-serotonin (5-HT) reuptake inhibitor (NSRI) marketed as an antidepressant in Japan and Europe. Previous studies performed by Bymaster and Desfield-Ahmad (1999) have demonstrated that MIL preferentially blocks the reuptake of NE over that of 5-HT (Mond, Charveron et al. 1983). A pattern opposite to that seen by 5-HT-NE reuptake inhibitors (SNRI) antidepressants, such as venlafaxine and duloxetine (DUL) (Kasamo, Blier et al. 1996; Roseboom and Kalin 2000; Bymaster, Desfield-Ahmad et al. 2001). Other studies suggest that MIL is also a low-affinity N-methyl-D-aspartate (NMDA) antagonist (Shuto, Takada et al. 1995). These properties are similar to those of some tricyclic antidepressants (TCAs), particularly amitriptyline and its major metabolite, nortriptyline (Cai and McCaslin 1992; Eisenach and Gebhart 1995). However, MIL is nontricyclic in structure and lacks anticholinergic and antihistaminergic activity (Moret, Charveron et al. 1985; Mochizuki, Tujila et al. 2002).

The goals of the present study were to (1) confirm the unique reuptake ratio of milnacipran with expressed human 5-HT (hu SERT) and NE (hu NET) transporters, and (2) determine the site of action of MIL at the NMDA receptor. DUL was used as a comparator drug.

Methods

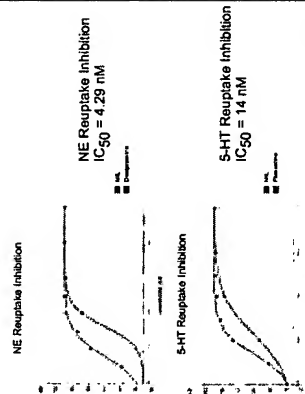
- Drugs**
- Milnacipran was obtained from Parke-Davis (Ann Arbor, MI).
 - Duloxetine was synthesized by Cypress Bioscience (Cypress, CA).
- Assays**
- All assays were performed by Cypress Bioscience (Cypress, CA).
- NET Binding**
- NET binding was determined by [³H]-Methylphenidate (MPH) binding to NET in rat brain synaptosomes (Gall et al. 1992).
 - Binding curves were generated from inhibition binding data obtained at 5 concentrations (10 nM, 30 nM, 100 nM, 300 nM, 1000 nM) of MIL.
- DUL Binding**
- DUL binding was determined by [³H]-DUL binding to NET in rat brain synaptosomes (Gall et al. 1992).
 - Binding curves were generated from inhibition binding data obtained at 5 concentrations (10 nM, 30 nM, 100 nM, 300 nM, 1000 nM) of MIL.
- NET Reuptake Inhibition**
- NET reuptake inhibition was determined by [³H]-Methylphenidate (MPH) uptake into NET-expressing cells (Gall et al. 1992).
 - Reuptake curves were generated from inhibition binding data obtained at 5 concentrations (10 nM, 30 nM, 100 nM, 300 nM, 1000 nM) of MIL.
- 5-HT Reuptake Inhibition**
- 5-HT reuptake inhibition was determined by [³H]-5-HT uptake into 5-HT-expressing cells (Gall et al. 1992).
 - Reuptake curves were generated from inhibition binding data obtained at 5 concentrations (10 nM, 30 nM, 100 nM, 300 nM, 1000 nM) of MIL.
- NMDA Receptor Binding**
- NMDA receptor binding was determined by [³H]-Methylphenidate (MPH) binding to NMDA receptors in rat brain synaptosomes (Gall et al. 1992).
 - Binding curves were generated from inhibition binding data obtained at 5 concentrations (10 nM, 30 nM, 100 nM, 300 nM, 1000 nM) of MIL.

MIL Displays Higher Affinity for huSERT than huNET



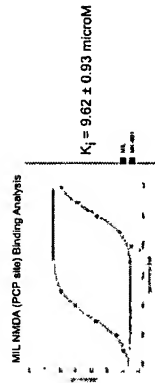
MIL's NE:5-HT binding affinity ratio of 1:9 is almost identical to that of duloxetine (1:9.4; Bymaster, Desfield-Ahmad et al. 2001).

MIL Preferentially Blocks the Reuptake of NE vs. 5-HT



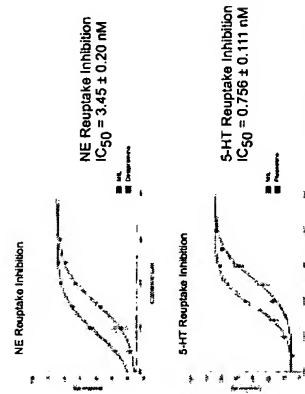
MIL's ratio of reuptake inhibition of 3.3:1 (NE:5-HT) is reversed compared to that of its binding affinity (1:9, above).

MIL is an NMDA (PCP site) Antagonist, Unlike DUL



The binding affinity demonstrated above is similar to that observed for the binding of MIL to the PCP site (Kasamo, Blier et al. 1996; Eisenach and Gebhart 1995). Note that MIL showed no appreciable binding for the NMDA 5-HT polyanionic sites. DUL showed no appreciable binding at any of the NMDA receptor sites tested.

DUL Preferentially Blocks the Reuptake of 5-HT vs. NE



DUL's ratio of reuptake inhibition of 1:4.8 (NE:5-HT) is reversed compared to that of MIL, but consistent with its binding affinity (Bymaster, Desfield-Ahmad et al. 2001).

Conclusions

- MIL is an NSRI against human 5-HT and NE transporters, thus extending the previous rodent work.
- MIL's binding affinity ratio for 5-HT and NE is inverted compared to that of its reuptake inhibition ratio.
- We confirm that DUL is an SNRI
- Finally, MIL is a low affinity PCP-site NMDA antagonist, unlike DUL.

The combination of NE>5-HT reuptake inhibition and low-affinity NMDA PCP-site antagonism resembles certain tricyclic antidepressants (TCAs), particularly amitriptyline/nortriptyline. Moreover, this combination of activities may make MIL ideally suited for the treatment of certain chronic pain conditions that are associated with affective disorders, including fibromyalgia, irritable bowel syndrome, and certain subsets of low-back pain (Kranzler, Gendreau et al. 2002; Rao 2002).

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